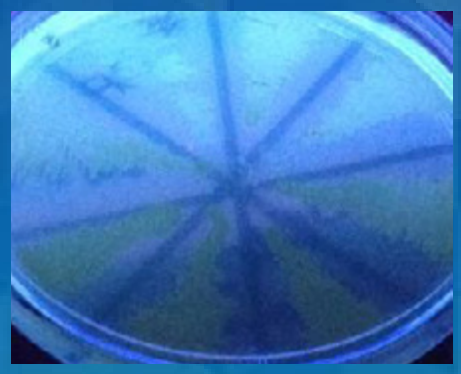
PARTS REGISTRY HANDBOOK

A GUIDELINE INTO iGEM'S STANDARDIZED PARTS



VIRTUS PARVA

TEC-CEM

FOREWORD

Working with the manuals became and inspiring piece of work. Because the work put into them was, primarily, in order to help and assess new teams and experienced ones as well. Several parts became involved in the creation of these materials, as the original holders of the idea, the ITESM campus CEM team knowing this would not be an easy walk, sought co-authorship. That's when the Virtus Parva team decided to become involved and help each other out. There are three manuals, each, with a specific area: the Good Laboratory Practice manual, which helps on setting the standards of the work done in the laboratory; the iGEM Registry manual, which is an easy to follow step guide for all those who venture into the iGEm parts domain, it covers from looking up a standard part into adding your own, going through the sending of a part for sequencing and the shipping process; and last but not least, the iGEM team manual, this one hopes to cover most of the team formation process, as well to be a guideline for teams to follow along the process until the Jamboree is concluded. This work was the most complex to resolve, still, some teams may not find it insightful since there are many ways a team responds and stays motivated.

This manual will take you into the world of iGEM's Standardized Biological Parts known as BioBricks, and show the importance they have within the competition. In this work, you may find a quick guide into the browsing, adding and requesting of these biological components.

CONTENTS

The Registry Manual

iGEM works continually in order to enhance and promote knowledge regarding synthetic biology, that is where the standardization and categorization of biological parts plays a crucial role into iGEM's goals. The parts registry is an encyclopaedia where all the submitted BioBricks from previous competitions are gathered to browse and research, or the case being, request them in order to work with these standard parts.

The iGEM registry is evergrowing as participants continually add new parts into the catalog, as well as the inclusion of additional information into BBs they are currently working on. On this document, a brief step-by-step guide will be provided for teams working with the feature.

Build on Standard Biological Parts

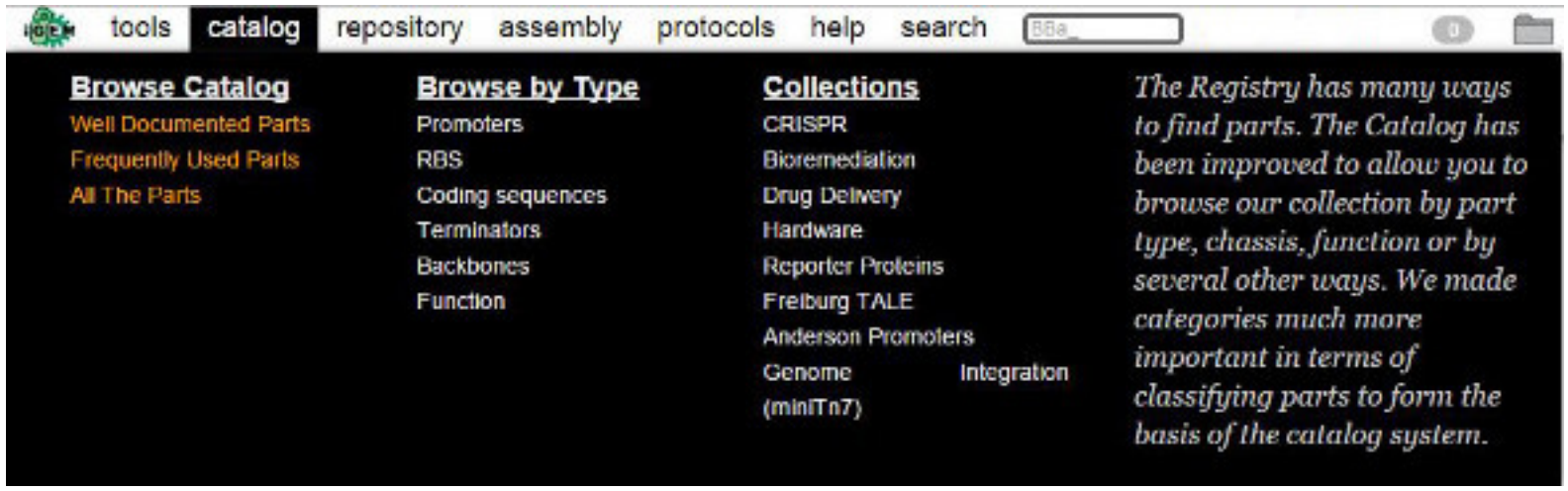
Every part must be standardized which means having a prefix and suffix (restriction sites) flanking the beginning and end of the part samples that will allow them to be assembled to form a new part. A standardized part can be removed from the plasmid backbone to be used in another operation like assembly, measurement, or shipping.

How to search in the Registry?

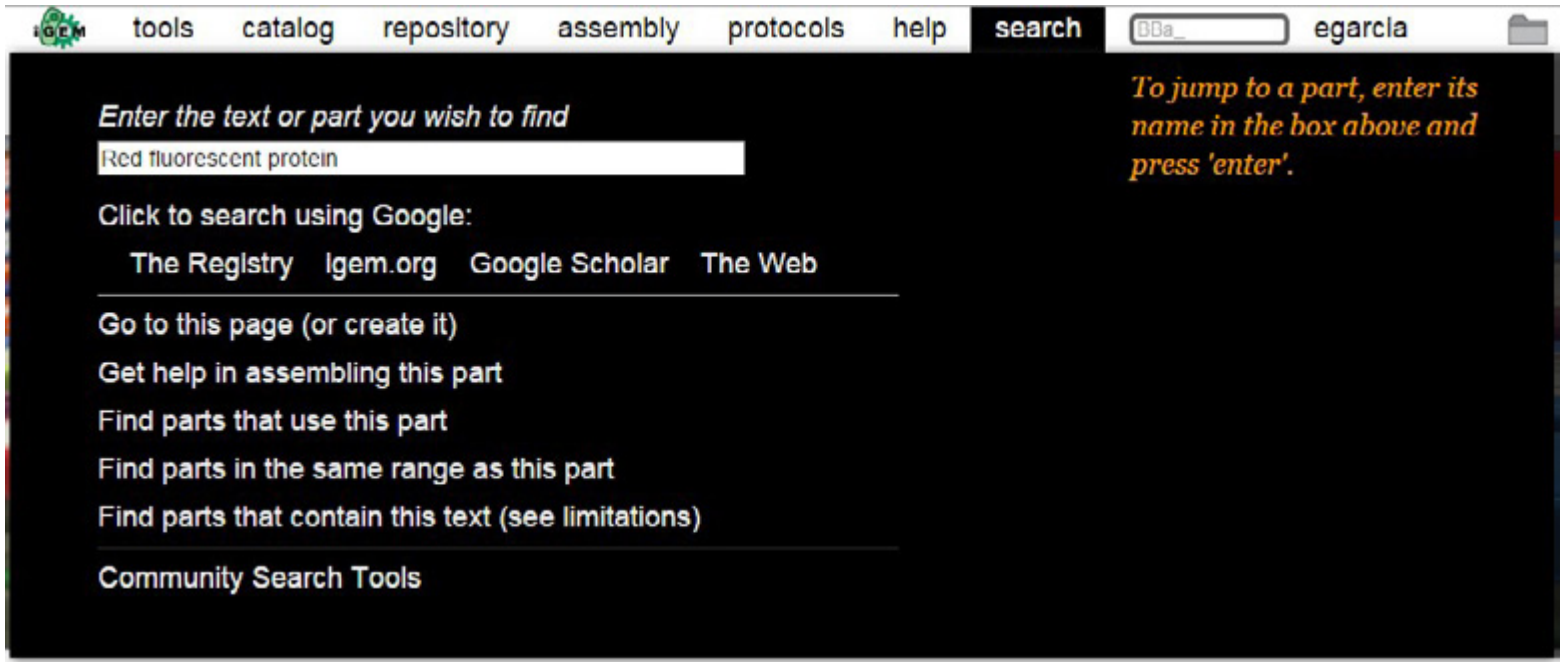
If you know the biobrick name (BBa_XXXXXX):

1) Write the name of the BioBrick in this space 

and go directly to the part site.
2) You can just select the gray triangle beside the space for searching and look for other type of information like "get help in assembling this part" or searching also in google.
3) Another option is selecting the catalog menu to see all the parts classified by part type.



If you don't know the part:
Click on SEARCH in the registry tools. Type any key word or the name of a gene, protein, enzyme, etc., as it is shown on the image below.

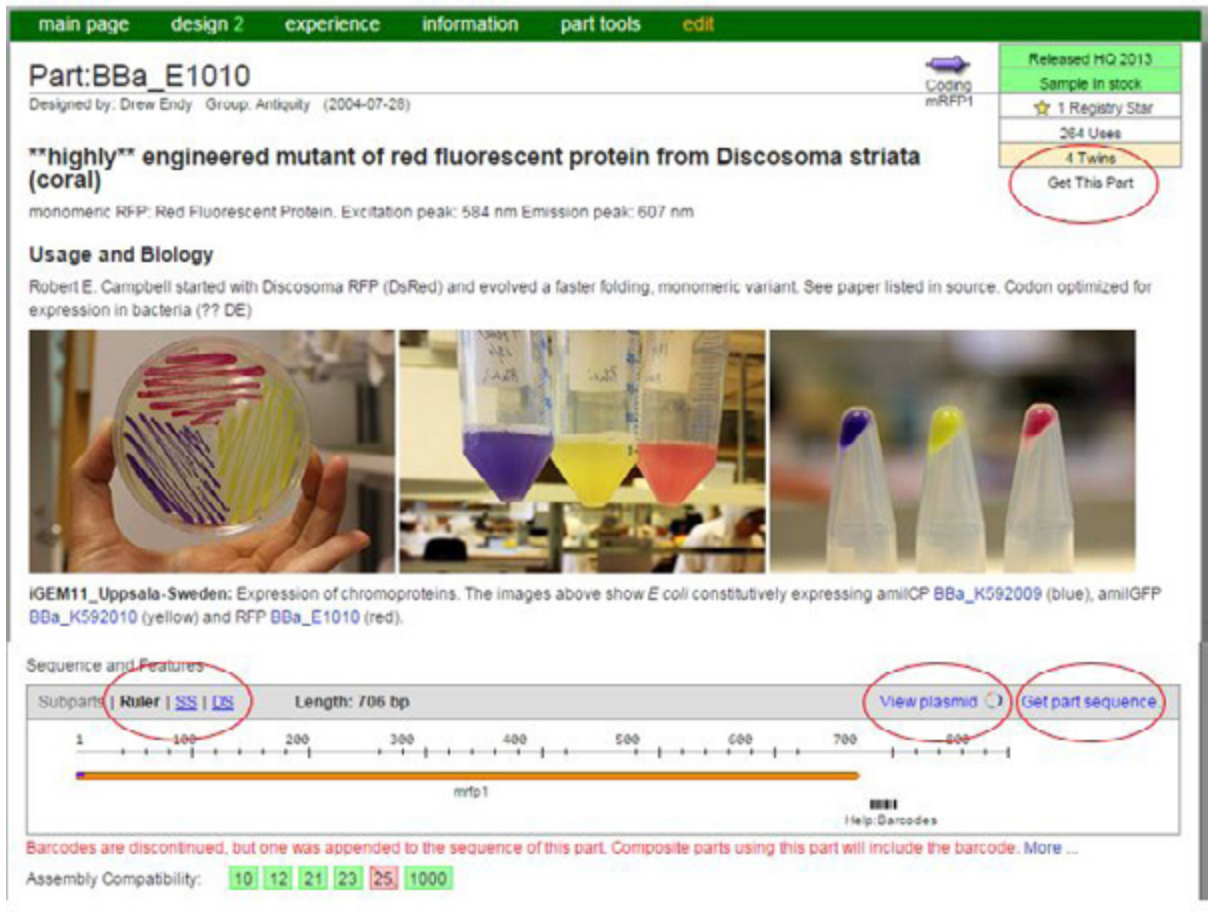


Now you have different options to search by clicking on the following:

1) A general search using google in the registry, igem.org, Google scholar or the web.
2) An specific page (Go to this page) from parts.igem.org.
3) If you have a biobrick assembly problem you can click on Get help in assembling this part.
4) Browsing parts that use the searched part, in this case, the key word searched, click on Find parts that use this part.
5) To search similarities between the written keywords and biobricks registered, click on Find parts in the same range as this part.
6) To have a search of biobricks that contains the keyword(s) typed, click on Find parts that contain this text.

Part Main Page

Once you found an interesting part, you can check all the part's documentation, at the top of the page or on the right side.



Clicking on view plasmid in the bottom of the page you will find a plasmid map which contains the searched biobrick. You can analyze how it looks on a plasmid.

Clicking on Get part sequence you will find the biobrcik sequence without prefix and suffix in Fasta format. So you can copy and paste it into an analysis software, such as serial cloner®, Snap Gene® or another favorite software.

In SS option you can see all the biobrick attributes in a single stranded sequence, while in DS option you can be able to see them on a double stranded way.

The **get this part button** would be explained in the following sections of this handbook.

Requesting Parts

in some cases, more parts than the ones provided in the distribution kit will be needed, those can very well be requested from the Repository. To check availability of such parts, it is necessary to pay attention to the Part Status Box that accompanies the Bio Part.

Part Status Box

The **Part Status Box** appears in the top right of a part's page. It includes:

- the status of a part's sample(s): *Sample In Stock / Sample It's Complicated / Sample Not In Stock*
- a link to the part's Get This Part page: where you can find the location of a sample in the current distribution, or in the Repository

Sample In Stock

Released HQ 2013
Sample in stock
Experience: None
0 uses
Get This Part

- There is a **sequence confirmed or long part** sample in stock in the Repository
- It does **not** guarantee that the sample is available in the current distribution
- You can request a sample

Sample It's Complicated

Not Released
Sample Pending
Experience: Works
0 uses
Get This Part

- There is a sample in stock in the Repository, but it is **not** sequence confirmed
- The sample may have poor quality control results (**Partially Confirmed, Single Error, Inconsistent, Bad Sequencing**) or no quality control information
- You can request a sample

Sample Not In Stock

Not Released
Sample Not in stock
Experience:
0 uses
Get This Part

- The Registry does not have a sample in stock
- This part **cannot** be requested from the Registry

Availability

After finding the part you were looking for, go and click the Get This Part button. A new page will open and from there you will know if your part sample is included in a current Distribution Kit (and its location, plasmid backbone, and sequence result), or if there are other samples for the part in the Repository and their location

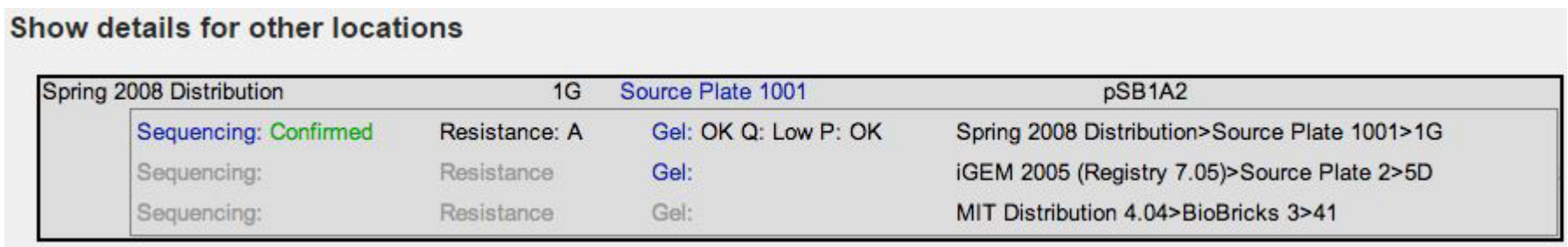
Sample Available in Distribution

Part BBa_C0040 is available in these Registry distributions: Tree View Show details for 22 locations

Distribution	Well	Plate	Plasmid Backbone	Sequencing	Well Id	
Spring 2013 Distribution	4A	2013 Kit Plate 5	pSB1A2	Confirmed [U]		More...
Spring 2013 Distribution	1O	2013 Kit Plate 3	pSB1C3	Confirmed		More...

The table will tell you, too, if the part you are looking for can be acquired by transforming any of the parts included in the wells kit. When the part required is not in the Distribution kit, a window like this will pop up.

To navigate amongst the distinct locations of the wanted part, simplu click the **Show details for (x) Locations.** After this, a new window will appear, in it you'll find a new table, similar to this one:



Please, keep in mind that not every sample has quality control information, but look for those with confirmed sequencing status. In case you do not find a Confirmed Sequence, you can still work with those that haven't been so.

Requesting

Requests are made via email at: hq@igem.org.
In them, don't forget the following format:

Subject: "Part Request: [Your Team/Lab's Name]."
Part Name (BBa...)
Plasmid Backbone (as documented on the Get This Part page)
Sample Location: source plate and well
Quality Control Results: sequence and gel results (if available)

Once a request is made you should be contacted by iGEM HQ within two business days.

Shipping

Last but not least, is the shipping process. Be sure to fill appropiately for every bit of data asked, be it adress or for customs purposes. The time of arrival may vary, but don't let the anxiety win this one, the part is almost home.

Shipping Times

For shipments within the United States shipping times are generally no more than two days through UPS. For international shipments, shipping times through UPS can differ by a number of days depending on the receiver's address.

A confirmation email with a tracking number for your shipment will be sent to you when your part request is ready to be shipped. You can then track the location of your shipment.

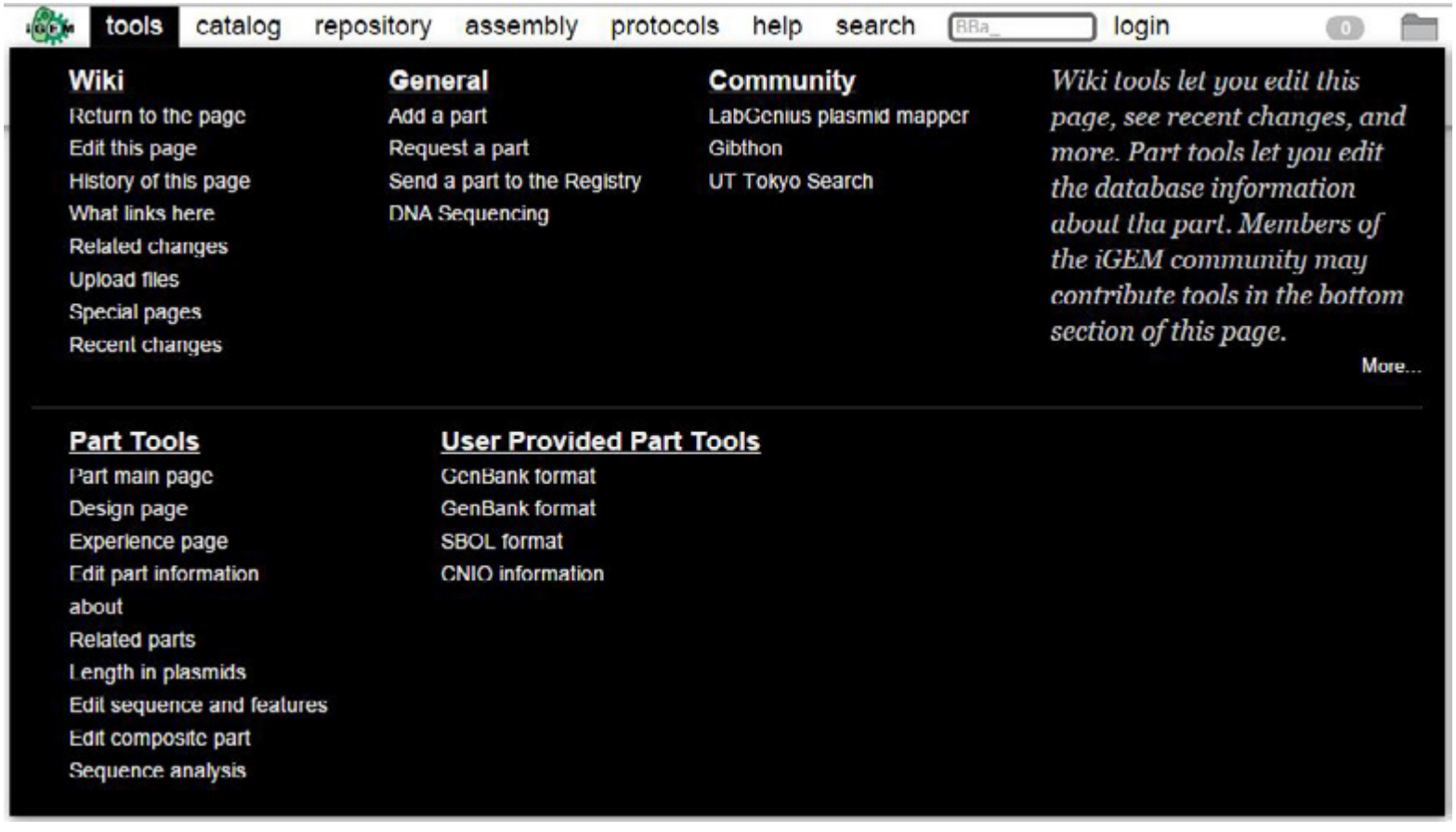
Agar Stabs

We send out part requests as agar stabs (LB agar). The shelf life of these are short, so it is best to plate from the stab as soon as possible

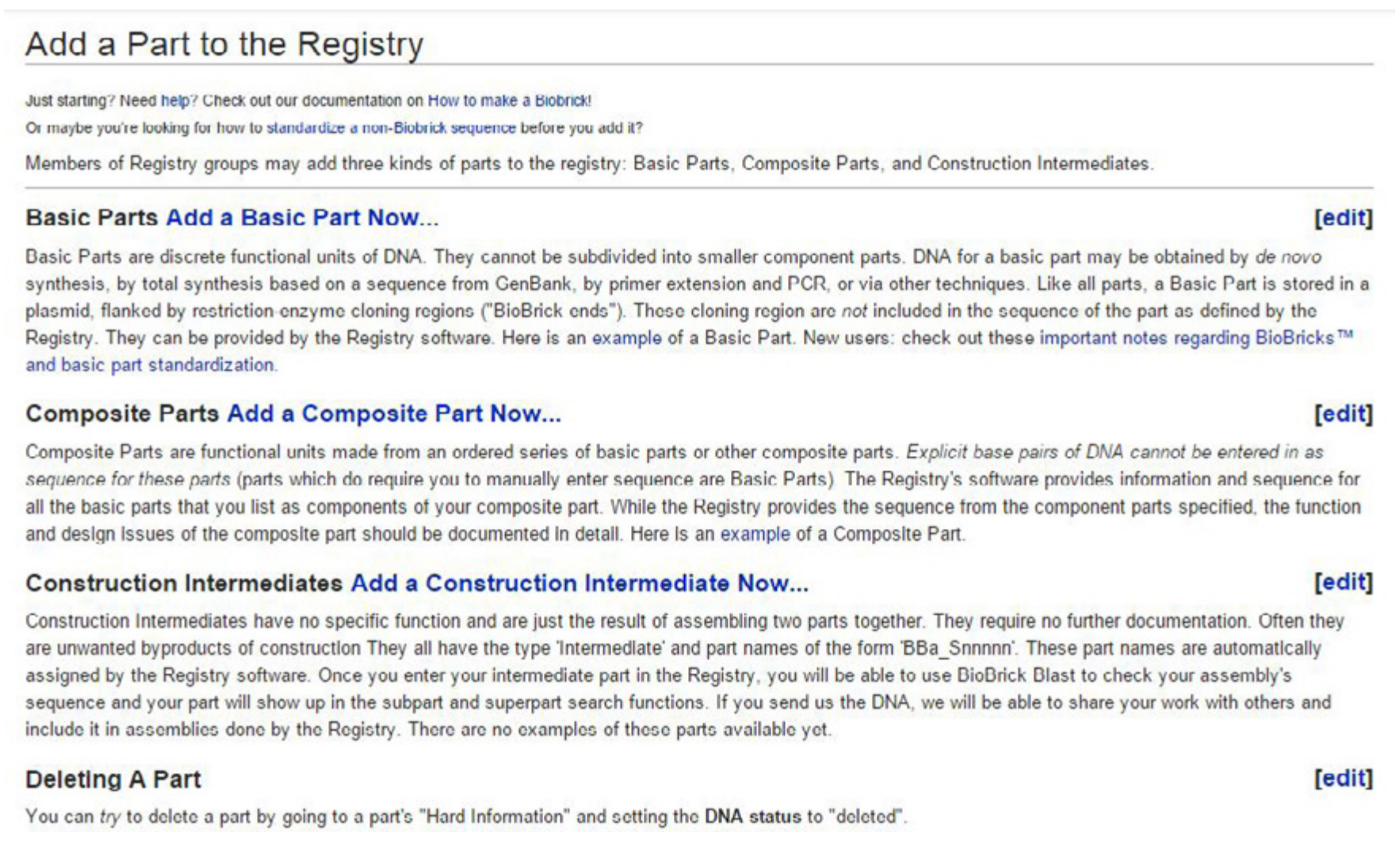
- The agar will have a hole from when it was stabbed. Dip an inoculating loop into the stab, and plate onto a petri dish of LB agar with the appropriate antibiotic.
- Incubate the dish overnight at 37C (14-16hr)
- Pick a single colony to start up a culture
- Miniprep to extract plasmid DNA
- Use the part!

Add a Part

If you are ready to add your own contribution, you should check the next steps:
First go to the section: **tools** in the iGEM main page.

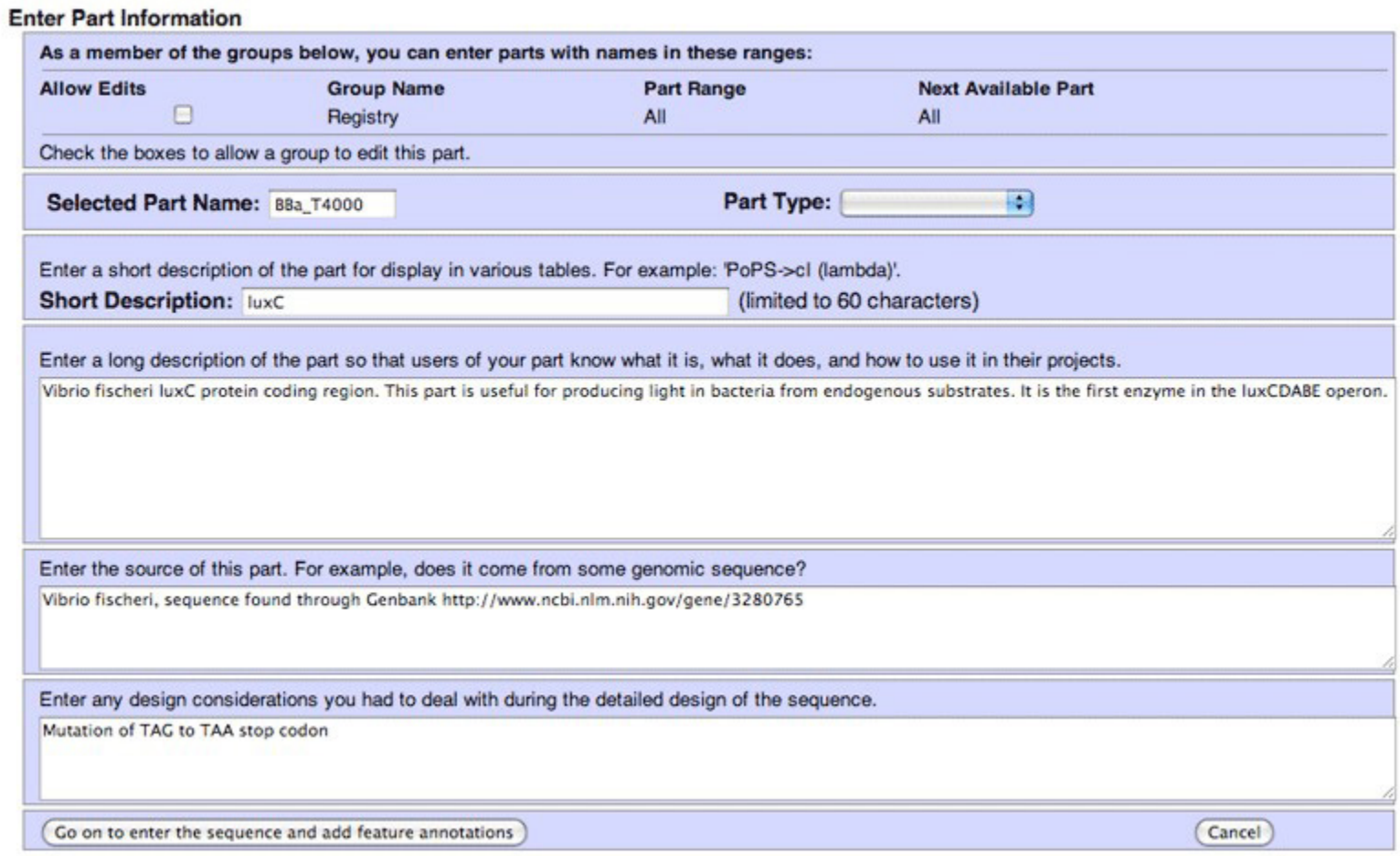


THEN, CLICK ON THE **ADD A PART** OPTION OR **SEND A PART TO THE REGISTRY** IN THE GENERAL SECTION.
IF YOU CHOSE TO ADD A PART, A NEW WINDOW WILL OPEN UP:



ON THIS OCCASION, WE WILL ADD A BASIC PART, IN ORDER TO ENTER THE PART, YOU ARE GOING TO NEED SOME INFOR-

mation about the source, design, function, and composition of the part. Left click on the **Add a Basic Part Now** option, this is the form your team will have to fill out:



Group: Select groups that will have permission to edit your part.
Part Name: Select your part name within your assigned part range.
Part Type: Classifications show here:

Promoters: A promoter is a DNA sequence that tends to recruit transcriptional machinery and lead to transcription of the downstream DNA sequence.

Ribosome Binding Sites: A ribosome binding site (RBS) is an RNA sequence found in mRNA to which ribosomes can bind and initiate translation.

Protein domains: Protein domains are portions of proteins cloned in frame with other proteins domains to make up a protein coding sequence. Some protein domains might change the protein's location, alter its degradation rate, target the protein for cleavage, or enable it to be readily purified.

Protein coding sequences: Protein coding sequences encode the amino acid sequence of a particular protein. Note that some protein coding sequences only encode a protein domain or half a protein. Others encode a full-length protein from start codon to stop codon. Coding sequences for gene expression reporters such as LacZ and GFP are also included here.

Translational units: Translational units are composed of a ribosome binding site and a protein coding sequence. They begin at the site of translational initiation, the RBS, and end at the site of translational termination, the stop codon.

Terminators: A terminator is an RNA sequence that usually occurs at the end of a gene or operon mRNA and causes transcription to stop.

DNA: DNA parts provide functionality to the DNA itself. DNA parts include cloning sites, scars, primer binding sites, spacers, recombination sites, conjugative tranfer elements, transposons, origami, and aptamers.

Plasmid backbones: A plasmid is a circular, double-stranded DNA molecules typically containing a few thousand base pairs that replicate within the cell independently of the chromosomal DNA. A plasmid backbone is defined as the plasmid sequence beginning with the BioBrick suffix, including the replication origin and antibiotic resistance marker, and ending with the BioBrick prefix.

Plasmids: A plasmid is a circular, double-stranded DNA molecules typically containing a few thousand base pairs that replicate within the cell independently of the chromosomal DNA. If you're looking for a plasmid or vector to propagate or assemble plasmid backbones, please see the set of plasmid backbones. There are a few parts in the Registry that are only available as circular plasmids, not as parts in a plasmid backbone, you can find them here. Note that these plasmids largely do not conform to the BioBrick standard.

Primers: A primer is a short single-stranded DNA sequences used as a starting point for PCR amplification or sequencing. Although primers are not actually available via the Registry distribution, we include commonly used primer sequences here.

Composite parts: Composite parts are combinations of of two or more BioBrick parts.

•Short Description: Technical or biological function of the part.
•Long Description: Detailed description of the part, function and requirements.
•Source of this part: The origins of the sequence
•Design Consideration: If you needed to change the part with mutations or codon optimization you must describe it in this section.
Now, click on **Go to Enter the sequence and features annotation**

Sequence and Features

Sequence and Features

Your part has been added to the Registry, but only its name has been designated along with some descriptive information. Now you will need to add its sequence and diagram its features. You can always return to this page by going to **Tools > Edit Sequence and Features.**

Add your part's sequence

- Click on **Edit**, copy & paste your sequence into the editor,
- and **Save**!
- Remember, do not include any standard's prefix or suffix.

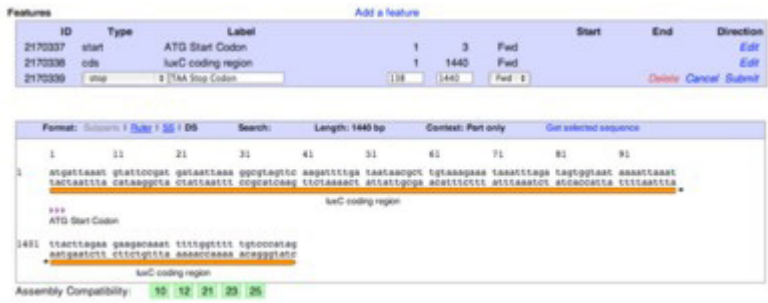
Once your sequence has been saved...

- you will be able to add features to your part.
  - **Ex.** Three features have been added to BBa_T4000, to show its start codon, its cds, and its stop codon.
- the **Assembly Compatibility box** will also check if your part's sequence meets the requirements of the Registry-supported standards.
  - A green box means that your part is compatible with that standard
  - A red box means that your part has an illegal restriction site for that standard. Hover over it to find out where these restriction sites are.
    - You can use this information to determine if you'll need to mutate these restriction sites out to ensure that the part is compatible with your preferred standard.
- **Your part must be BioBrick compatible**

A part is BioBrick compatible if it does not include illegal sites: EcoRI, SpeI, XbaI, PstI y NotI. because they belong to the suffix and prefix of the BioBrick assembly standard. Therefore, all parts submitted should not carry any of these sites. If they do, they will be deemed incompatible and rejected for any contribution in the registry.

Now that the part has been described, the sequence should be added. Write the sequence into the editor, sans prefix or suffix. Then you can save the sequence.

Now features can be added. Features include start codon, stop codon, etc.
Also in this space, the part will be either identified as BioBrick compatible or not. Consider that this information might be a guideline in case you need to mutate your part to make it compatible.



Hard Information

This information helps classify and organize the part in the Registry and database.

Page Header

Part Name	**BBa_T4000**	*Edit*
Short Description	**luxC**	*Edit*
Part Type	Coding	*Edit*
Nickname		*Edit*
Designer(s)	**Vinoo Selvarajah**	*Edit*
DNA Status	Planning	
DNA is only defined as Available if it is in the repository. How to send parts.		
Qualitative Experience		*Edit*
Group Favorite	No	*Edit*
Star Rating	None	*Edit*
Delete This Part	Not Deleted	*Edit*

- Part Name: Its official name
- Part Type: select the type of the part.
- Short Description: biological or technical description of the part's function
- Nickname: The nickname of the part.
- Designers: People who collaborated in the part's design. The register's name will be listed by default, but more designers can be listed here.
- Dna Status: Deleted, Planning, Sent, Available, etc. Registry edited.
- Qualitative Experience: Indicates if the part works
- Group Favorite: You can choose it as a favorite if you love it.
- Star Rating: Decided by Registry
- Delete Part: You can delete it from the Registry

Page Footer

Parameters

This section allows for technical specification. You can specify the type of part and the organism it is derived from.

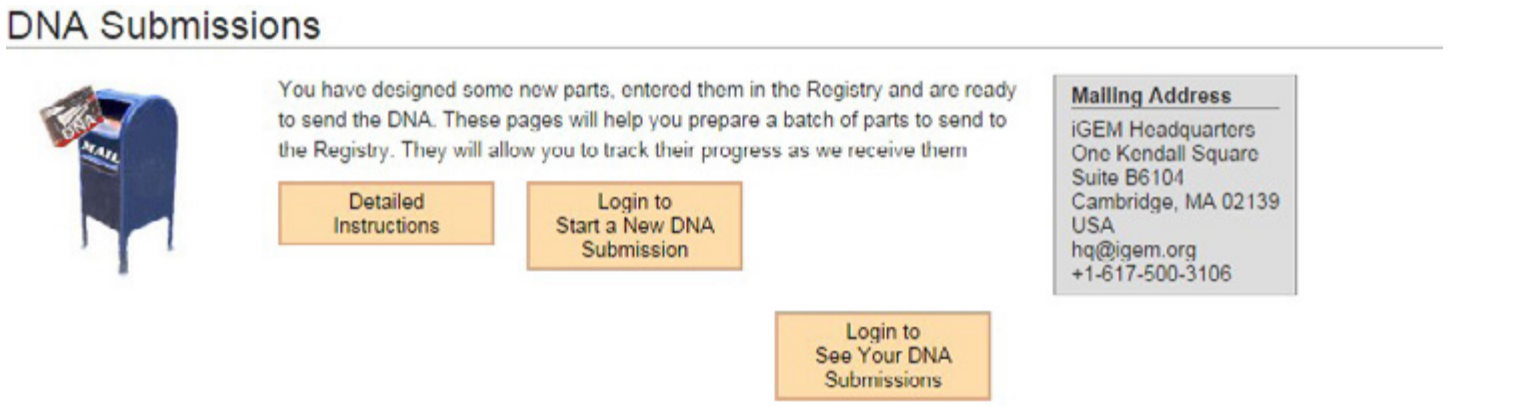
Categories

Allow for a part to become content in automatically generated part tables, which is important in defining the organization of your part within the Registry, and specifically for the Catalog of Parts and Devices.

Sending A DNA Part Submission

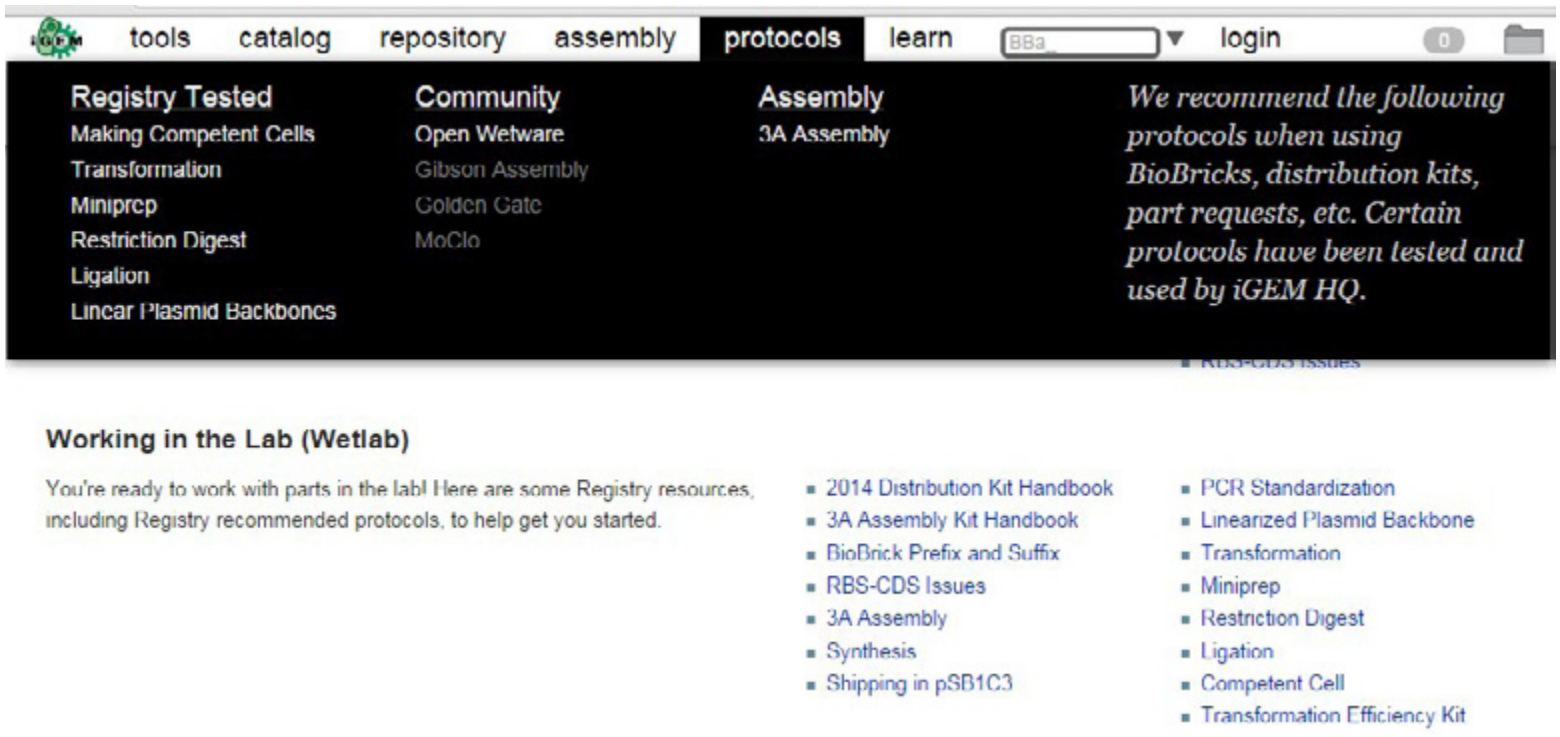
1) BioBrick compatible.
2) The sample must be in pSB1C3 because it is a high-copy plasmid which improves miniprep yields for parts and users can get parts from this plasmid to use into another plasmid.
3) Documentation: All parts shipped to the Registry must be added first. You must consider that it is always better to send few parts well documented than sending several that are not.

Submission Formats



Protocols

The registry also includes some protocols and information to help you start with the wetlab:



Composite Parts Registry

Now, into the Composite Part, adding, which is not too stray off the Basic Part addition

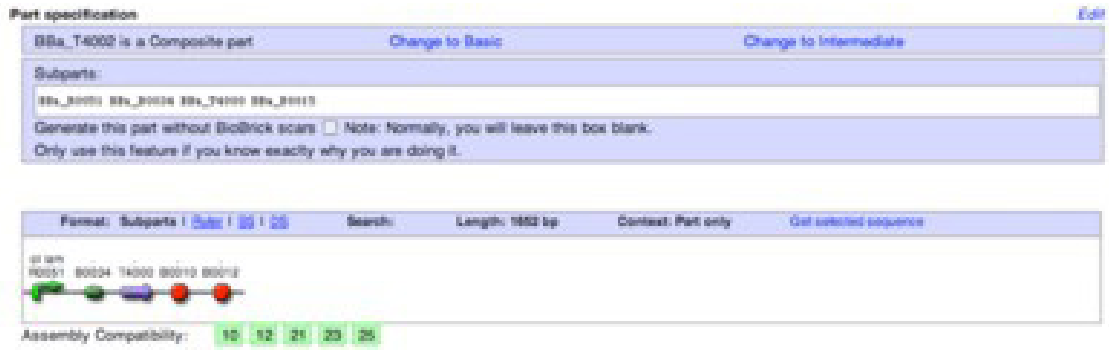
1. "Tools" in the Registry menu
2. Add a Part
3. Add a Composite Part Now...

Enter Part Name and Information

- Group: select groups that can edit the part
- Part Name: enter a part name. Must not be already in use.
- Part Type: select the type of the part.
- Short Description: biological or technical description of the part's function
- Long Description: Detailed description of the function, as well as requirements
- Source: enter the source. It can be the source of the sequence.
- Design considerations: enter any considerations, like mutations, elimination of restriction sites, optimization, etc.
- Subparts: You can add the basic or multiple parts that make up your part.

Sequence and Features

Here you can edit any mistakes on the list of subparts described in the previous section.
Unlike basic parts, here a sequence cannot be entered. Instead, the Registry will use the provided information to put the part's sequence together.



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Parameters

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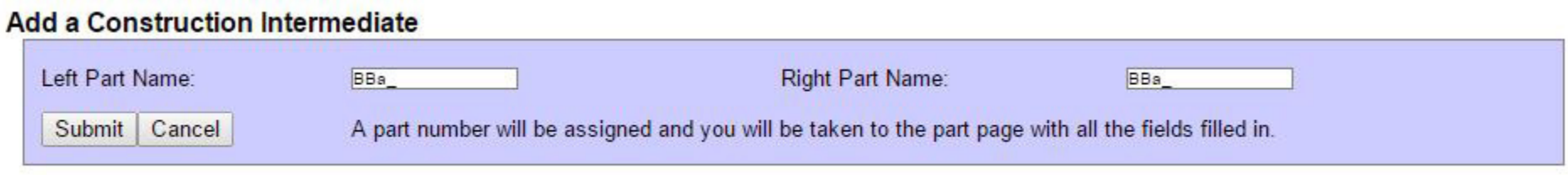
Construction Intermediates

The construction intermediates are the byproduct or inbetween steps of distinct sequencings, they are unwanted specimens that do not require thorough information input. Thus, it is only necessary to add the left and the right extremes of the component in order to register it into the system. Deep analysis of an intermediate can be assessed by sending it to the headquarters and get the deciphering of the sequence ignited.



References

http://parts.igem.org/